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### How Much Is Enough? Ethical Consideration for the Depletion of Large Public Cord Blood Units (CBU)

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**Background:** The Be The Match Registry® provides access to over 230,000 CBU with a median pre-freeze Total Nucleated Cell (TNC) count of 105 x 10^7 TNC. Prior studies have established an acceptable dose threshold of at least 2.5 x 10^7 for the treatment of hematological malignancies. Pediatric patients often only require a single CBU to achieve suitable cell dose for transplant (Tx) and some have received cell doses per patient weight in excess of 20 x 10^7 TNC/kg. Meanwhile adults may require multiple units to achieve the recommended minimal dose. This study evaluated whether CBU Tx in peds achieving a cell dose >20 x 10^7 TNC/kg deplete a unit that may be suitable for an adult patient when another potentially acceptable unit is available for the child.

**Methods:** We identified 74 single CBU Tx of patients age 0-12 years old, facilitated through the Be The Match Registry from Sept 2009 to Aug 2012 with a Tx cell dose >20 x 10^7 TNC/kg. The CBU searches were reviewed to determine whether another suitable CBU (10-20 x 10^7 TNC/kg and equivalent or better low resolution HLA-A, B, C, high resolution -DRB1) was available for that patient. A TNC threshold for CBU suitability for a potential adult patient of 178 x 10^7 was established based on the historical median weight of 71kg for adult CBU Tx recipients.

**Results:** Of the 74 Tx evaluated, 58 (78%) units had a minimum TNC of 178 x 10^7 (range 178-452), large enough for the median weight adult patient. In 48 of the 58 cases a suitable CBU with 10-20 TNC/kg was available on the search with an equivalent or better HLA match and 23% of the time the lower TNC CBU was a better HLA match. If the lower TNC CBUs had been selected for this cohort, the median cell dose would decrease from 28.7 to 11.5 TNC/kg.

**Conclusions:** The number of CBU in the registry that meet the median adult patient dose of 178 x 10^7 TNC is 16,494 (7%) CBU compared to 234,292 available for peds Tx. Transplant practice is often to take the largest CBU available for a patient, with consideration of HLA match differing between centers. This study shows that CBU used in Tx for children can exceed 20 x 10^7 TNC/kg. These CBU have a large TNC and could be suitable for adolescent or adult single cord transplantation. Although 74 CBU Tx correspond to a small proportion of total peds (age 12 and under) single CBU Tx during this timeframe (n=951), these units may offer the only opportunity for an adult patient. With a limited number of CBUs achieving high TNC available for adult patients, consideration of the ethics of providing a young patient with an adequate TNC CBU (e.g. 10-20 x 10^7 TNC/kg) vs the largest TNC CBU will continue to confront the community. Centers should consider selecting a CBU with smaller, yet still substantial cell dose, particularly when it's a better HLA match. Future outcomes research is needed to elucidate the optimal TNC or identify a maximum threshold recommendation for guidance in CBU Tx in small children prior to a policy being implemented.

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### Hematopoietic Stem Cell Transplantation for Sickle Cell Anemia with Busulfan-Based Reduced Intensity Conditioning: Cure and Fertility

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Hematopoietic stem cell transplantation (HSCT) is increasingly being used in the management of patients with sickle cell disease (SCD). Although cure remains the fundamental goal, preservation of fertility in this young patient population is an important consideration. While it is known that standard busulfan-based myeloablative conditioning uniformly causes permanent gonadal dysfunction, complete graft rejection is a serious drawback in busulfan-free exclusively immunosuppressive-based preparative regimens with fludarabine and cyclophosphamide alone. Therefore if busulfan is to remain a central part of the conditioning, a significant dose reduction may be necessary to cure and yet preserve fertility. The subsequent successful outcome and preservation of fertility in two young adults transplanted in our center with busulfan-based targeted-dose reduced intensity conditioning (RIC) for paroxysmal nocturnal hemoglobinuria, prompted us to consider this regimen in SCD. Between September 2008 and April 2012, 16 patients with SCD underwent HSCT in the Sultan Qaboos University Hospital, Oman. The conditioning regimen consisted of fludarabine 30mg/m2/day for 6 days, two days of targeted-dose intravenous busulfan (target $C_{ss}$ 850ng/ml) and rabbit anti-thymocyte globulin (10mg/kg/day for 4 days beginning on day minus 4). The median age was 18 yrs (range 9-40 yrs) and transplant indications were recurrent vaso-occlusive crises, acute chest syndrome and cerebrovascular events. There were nine males and seven female patients. The stem cell source was peripheral blood in 14 (88%) and bone marrow in 2 patients (12%). Fourteen patients (88%) are considered cured with a median follow up of 24 months (6-49 mths). Nine (64%) of these patients have complete donor chimerism (DC) while five (36%) patients have stable mixed chimerism ranging from 67-90%. Two patients (12%) rejected their grafts with rapid loss of DC by 6 months and are alive with their original SCA manifestations. Acute graft versus host disease (Grade III) occurred in only one patient and resolved with therapy. Of the 6 post pubertal female patients, only one has resumed normal periods following the transplant. The five others (median age 24yrs, range 16-27yrs) developed amenorrhea post transplant with high FSH and LH levels and have been started on hormonal replacement therapy. No tests of gonadal function in the male patients are available. Busulfan-based RIC HSCT is curative and appears to be safe even in older patients with SCA but gonadal damage continues to remain a long term complication. There may be a window of opportunity to further reduce busulfan exposure in an attempt to preserve

fertility. If such a strategy is used, novel measures to induce immune tolerance may also be required to minimize rejection.

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### Reconstitution of Lymphocyte Subsets and Outcomes After Matched and Mismatched Hematopoietic Stem-Cell Transplantation

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Allogeneic stem-cell transplantation (ASCT) is curative for many malignant and nonmalignant hematological disorders. We aimed to study reconstitution of lymphocyte subsets after matched and mismatched transplantation.

Lymphocyte subsets were evaluated by flow cytometry at 1,3,6 and 12 months (mo) post-ASCT. Lymphocyte recovery was determined using means at each time point and group differences assessed using analysis of variance.Time-to-event outcome were estimated by Kaplan-Meier survival curves and the log-rank test was used to evaluate differences between groups. 100 patients (pts) were included in the study: 25 received a matched sibling (MSD), 20 pts a matched unrelated donor ASCT 10/10 (MUD), 18 pts a 9/10 MUD, 9 pts a T cell depleted haploidentical (TCD haplo), and 28 a T cell replete haploidentical transplant (TCR haplo). 53 pts received bone marrow and 47 peripheral blood stem cells. Most patients were treated for acute leukemia (AML 41, ALL 23), 16 MDS, 6 CML, 4 CLL, 5 lymphoma. Median age was 43 years (range: 20-71). Median follow-up was 13.6 mo. 60 pts were alive and disease-free at last follow-up and 28 pts died 75% of relapse. Non-relapse mortality (NRM) was 6% for the entire cohort.

Overall, alive pts (vs. who died) had higher mean CD3 (615 vs. 349, $P = .03$ on day 90), CD8 (427 vs. 187, $P = .03$ on day 90), CD4 (391 vs. 54, $P = .01$, on day 365), and lower mean CD56 cells (178 vs 300, $P = .01$, on day 30) post ASCT. Pts who progressed (vs. did not), had lower 1 year mean CD4 (123 vs. 394, $P = .02$), lower mean CD3 (359 vs. 1147,$P = .06$), with no differences in CD8, NK, and CD45RA cells. NRM was associated with higher mean NK counts at 6 months (499 vs. 188, $P = .01$) and with lower mean CD3 at day 90 (184 vs. 557, $P = .07$). T-cell recovery occurred most rapidly in MSD transplants (Figure1), and interestingly, higher CD4CD25 cell numbers recovered early and most rapidly in the MSD transplants, which may partly explain a lower incidence of GVHD in this group. Overall, TCR haplos had a similar pattern of T-cell recovery and outcomes as 10/10 MUDs (Figure1). No significant differences in T cell subsets found between these two groups for CD3, CD4, CD8, CD45RA and CD4CD25 at any time-point. TCD haplos had most impaired T-cell reconstitution and outcomes (Fig. 1), characterized by early NK cell and delayed CD3, CD4, CD8 recovery. Interestingly, pts surviving 6-9 months post-transplant recovered CD3, C4, CD8 cells; however, naïve T-cell recovery was impaired for more than >1 year post-transplant, suggesting that T cell recovery comes predominantly from the memory T cell compartment.

In conclusion, recovery of lymphocyte subsets may vary widely with the type of transplant, may correlate with outcomes, and should be further explored post-transplant.

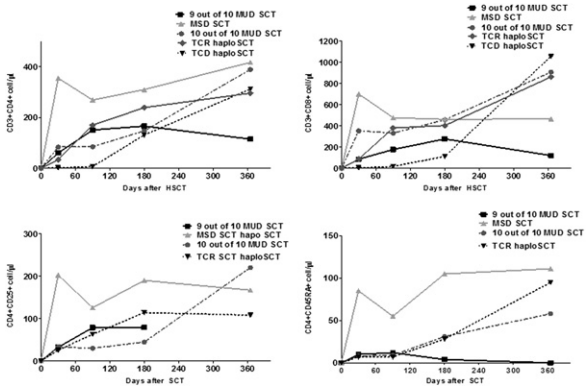


**Figure 1.** Recovery of T cell subsets after matched and mismatched ASCT

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### Immune Modulation of Antibody Responses Induced Through Allogeneic Cell Transplantation

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Combined renal and hematopoietic cell transplantation (HCT) protocols have successfully induced allograft tolerance despite loss of chimerism in patients; however, the mechanism remains unclear. Using a miniature swine model with demonstrated clinical relevance, we assessed immune responses following HCT in ten recipients that lost chimerism. All animals received 30 days of cyclosporine (CyA) with taper until day 45; low-dose total body irradiation (100cGy TBI) and T cell depletion using a CD3 immunotoxin (pCD3-IT). Six animals received mobilized donor cells and 4 animals received unmobilized cells. Control animals received allogeneic cells without any immunosuppression. Cellular responses were assessed by mixed lymphocyte reactivity and cell mediated lympholysis assays. Donor specific antibody was assessed by flow cytometry and complement mediated cytotoxicity assays.

Following loss of chimerism, anti-donor cellular proliferative and cytotoxic responses returned without alloantibody. Alloantibody responses were not induced even after a second exposure to donor cells intravenously without immunosuppression (n=10) or following donor skin graft rejection (n=3). Attempts to further immunize some of these animals (n=6) with multiple subcutaneous injections of donor cells with or without complete Freund's adjuvant also failed to induce donor specific antibody. In contrast, control animals exposed to donor cells without conditioning had sustained